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# Aspects of the Biology of Aeromonas Hydrophilia with Respect to the Striped Mullet, Mugil Cephalus L. in the St. Johns River

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# ASPECTS OF THE BIOLOGY OF *Aeromonas hydrophila* WITH RESPECT TO THE STRIPED MULLET, *Mugil cephalus* L. IN THE ST. JOHNS RIVER

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ABSTRACT

The aspects of the biology of Aeromonas hydrophila with respect to a striped mullet (Mugil cephalus L.) nursery were examined. A. hydrophila density in the natural water was found to be strongly correlated with turbidity and weakly correlated with water temperature. No correlations were found between A. hydrophila density and water depth, dissolved oxygen, pH, total alkalinity, specific conductivity, or phytoplankton chlorophyll$_a$ concentration. A. hydrophila density/g dry weight of stomach content were found to be correlated with chlorophyll$_a$ concentration/g dry weight stomach content. The survivability of striped mullet after capture was found to be primarily related to stress. Stressed striped mullet tended to become infected by A. hydrophila more readily. The mortality of transported striped mullet was reduced with the use of quinaldine, a fish tranquilizer, and by reducing crowding during transport.

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# I. INTRODUCTION

The striped mullet (Mugil cephalus L.), being a worldwide marine species, is important as a forage fish for marine and freshwater predators and is a popular bait for sport and commercial fishing. It is also an important commercial fish. The striped mullet fishery is a multi-million dollar commercial industry throughout the world. In Florida, the striped mullet is especially important as a bait and food fish. Additionally, the striped mullet has potential as a biological control agent for filamentous algae; the fish apparently controlled algae in a small pond in North Carolina (Grizzell and Neely, 1962) and appeared to be promising in experimental ponds in Central Florida (Osborne, 1980). In recent years outbreaks of red sore disease, which is caused by Aeromonas hydrophila, have been found in striped mullet populations throughout Florida (Fogt, 1980; Osborne, 1980). Striped mullet are prone to bacterial infection when handled and/or transported and red sore disease is suspected to be the major cause. This study was conducted to document the severity of A. hydrophila disease in striped mullet in the upper St. Johns River. The density of A. hydrophila in the St. Johns River and its relationship to the physicochemical and biological conditions of the river was evaluated.

History of Furunculosis Disease Caused by Aeromonas hydrophila

Furunculosis, hemorrhagic septicemia, or red sore disease is probably the most widely distributed and well known disease of wild and cultured fish in the world. The disease was first described in 1894 and the bacterium which caused the disease was isolated from diseased trout in a hatchery in Germany (Emmerich and Weibel, 1894). The disease was first reported in the United States in brook trout (Salvelinus fontinalis) in 1902 (Marsh, 1902). The disease has been reported in many other fishes including Micropterus salmoides (Hazen et al., 1978), Salmo gairdneri (Lallier et al., 1980; Trust and Sparrow, 1974 and Li and Flemming, 1967), Salmo trutta (Hosmer, 1980), Notemigonus crysoleucas (Meyer, 1964; Lewis and Bender, 1960), Dorosoma petenense (Haley et al., 1967), Dorsoma cepedianum (Rock and Nelson, 1965), Alosa sapidissima (Haley et al., 1967), Ictalurus punctatus (Rock and Nelson, 1965), Perca flavescens (Ross et al., 1960), Carassius auratus (Bullock, 1965) and various ornamental aquarium fish (Trust and Bartlett, 1974). The organism has been implicated in the red leg disease of frogs (Snieszko and Bullock, 1962) and is reported to be present in turtles and in the freshwater aquarium snail (Ampullaria sp) (Bartlett and Trust, 1976). In some instances, A. hydrophila has caused severe human infections (Ketover et al., 1973; Hanson et al., 1977; Deepe et al., 1980) and even human death (von Graevanitz and Mensch, 1968; Sanyal et al., 1975; Ketover et al., 1973).

## Etiology of Furunculosis Disease

Furunculosis disease results from an infection of a host by the bacterial agent, *Aeromonas hydrophila*. The epidemiology of the organism is not fully understood. This is further complicated by the occurrence of *A. hydrophila* infections in combination with the pertrichous protozoan, *Epistylis* (Hazen et al., 1978; Fliermans et al., 1977). There is disagreement as to the environmental habitat of the organism. Rippey and Cabelli (1980) stated that the presence of the bacteria appeared to be associated with the eutrophication (nutrient enrichment) of lakes and streams, while Hazen and Fliermans (1979) attribute the cosmopolitan distribution and facultative pathogenicity of *A. hydrophila* to its ability to survive in lotic, lentic, oligotrophic, mesotrophic and eutrophic freshwater and marine systems.

Many synonyms occur for *A. hydrophila*, these include *A. liquefaciens*, *A. punctata*, *Bacillus punctatus*, *B. rancida*, *Bacterium punctatum*, *B. salmomicida*, *Proteus hydrophilus*, *Pseudomonas granulata*, *P. hirudinis* and *P. punctata*. The bacterium is described as a gram-negative, asporogenous, motile (monotrichous polar flagellations) or non-motile rod which ferments several carbohydrates and produces a brown, water-soluble pigment when grown on the proper medium (Schubert, 1974; Ewing and Hugh, 1975). The disease condition is described as a generalized septicemia by Davis (1967) in which lesions, termed furuncles, may be produced if the disease does not progress too rapidly. When they occur, they

are formed as a result of the bacteria collecting in the capillaries where they reproduce rapidly causing destruction of the walls of the blood vessels. The bacteria progress into the muscle tissue where they continue to multiply, producing a swollen area containing the bacteria, blood and necrotic tissue. Filiermans et al. (1977) stated that fish infections generally result in scale erosion and sloughing, purulent lesions and bleeding of the fins. The gill capillaries may be congested because of clumps of bacteria. Internal congestion of organs, hemorrhagic lower intestine and petechiae in the peritoneum and musculature may be observed. Pathological changes include tissue necrosis in the kidney with the accumulations of bacteria in the glomeruli, a dark colored and enlarged spleen, petechaie in the muscles, integument, peritoneum and swimbladder and a flacid, inflamed intestine with a bloody discharge from the vent.

Several disease forms of furunculosis have been described and those given by Herman (1968) encompass some of the more common symptoms. He stated that the disease can appear in four different forms. First, in the acute form there is a sudden increase in mortality with few or no external gross symptoms (a rapid fatal septicemia with few gross lesions); second, in the subacute form there is a gradual increase in mortality with furuncules and hemorrhages at the base of the fins (with dropsy, blisters, abscessed areas, and scale protusion); the chronic form causes a low steady mortality with intestinal inflamation and variable

lesions (ulcerous form with furuncules and abscessed areas); and finally, the latent form does not cause mortality or symptoms, although the pathogen is present systemically. Snieszko and Axelrod (1971) attributed these changes to exotoxins and endotoxins produced by the bacteria. Osborne (1980) observed these symptoms in infected striped mullet captured from the St. Johns River and stocked into freshwater ponds. During his study, the infected fish appeared to be undergoing oxygen stress. The fish would swim erratically after approximately 12 hrs. They would surface and eventually beach themselves in the shallows along the shoreline. The body of the fish (back, belly, fins, lips) would appear as if bleeding had taken place through the skin and under the scales. These symptoms were common just prior to the death of the fish. Osborne (1980) concluded that the severity of the A. hydrophila disease in transported fish and the apparent lack of its cure made the striped mullet unacceptable for use as an algal control agent.

## II. METHODS AND MATERIALS

### Description of the Study Area

Two permanent study stations were selected on the upper St. Johns river in Seminole County, Florida. The stations were chosen because they represented the typical areas inhabitated by striped mullet. Underhill Slough (Station 1) was located in the northeast corner of Lake Harney while Cowhouse Slough (Station 2) was located approximately 3.7 km downstream from Lake Harney on the St. Johns River, Figure 1. The sampling stations usually experienced low water and a high algal biomass throughout an annual period. The water depth was usually less than 1.0 m at the sampling stations during the dry period of the year (November - March). The sediment at Underhill Slough (Station 1) was predominately sand. The aquatic plant community at this station was primarily dominated by alligator weed (Alternanthera philoxeroides Mart.), hydrilla (Hydrilla verticillata (L.f.) Royle) and coontail (Ceratophyllum demersum L.). Midway into the study, water hyacinth (Eichornia crassipes (Mart.) Solms.) became abundant from floating bonnets of vegetation entering the sampling station from flooded marshes adjacent to the station. Cowhouse Slough (Station 2) had a dark-brown silt bottom and was generally free of submersed vegetation.

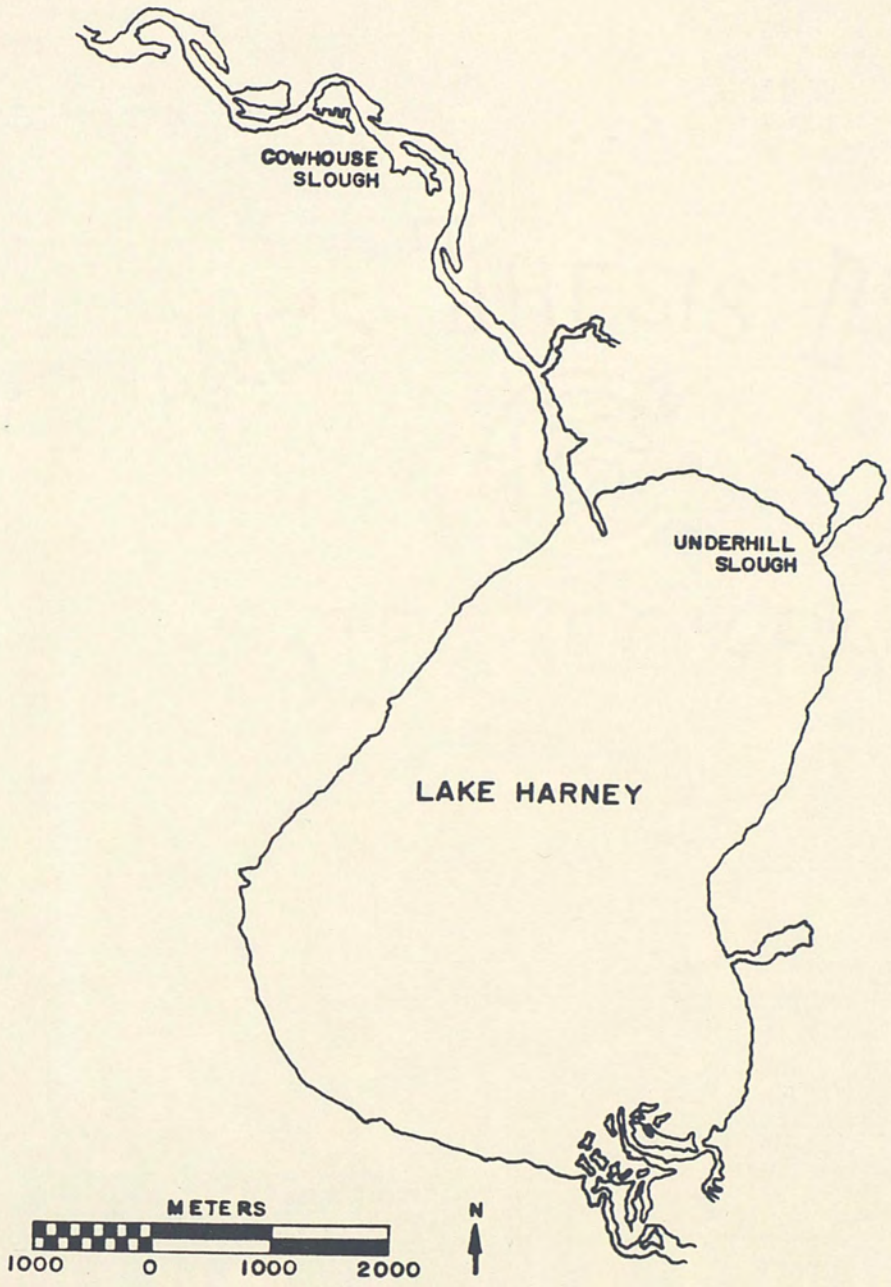


Figure 1. The location of Underhill Slough (Station 1) in Lake Harney and Cowhouse Slough (Station 2) on the St. Johns River.

Physicochemical Measurements

The physicochemical measurements were measured monthly in quadruplicate at each of the sampling stations. Monthly sediment samples and samples of the overlying water were collected in quadruplicate at each station to determine the chlorophyll$_a$ concentration and A. hydrophila density.

Water temperature and specific conductivity were measured with a Montedoro-Whitney SCT meter at the midpoint of the water column when the water depth was low and was taken at 0.5 m when the water depth exceeded 1 m. Turbidity was measured with the methods outlined in APHA (1975), while chlorophyll$_a$ concentration was determined spectrophotometrically with a Beckman Model 26 spectrophotometer according to the method of Richards and Thompson (1952). Dissolved oxygen was determined by the modified Winkler method (APHA, 1975). Total, bicarbonate and carbonate alkalinity were determined according to the methods outlined in APHA (1975).

Phytoplankton, Phytobenthos and Periphytic Algae Measurements

The chlorophyll$_a$ concentration of phytobenthic algae was determined with the Richards and Thompson (1952) method; the pigment was extracted in 90% acetone from predetermined wet weight sediment samples. The sediment samples were collected in 4.7 cm diameter plastic core liners that were previously disinfected with chlorox. The core liners were gently pressed by hand into the sediment in an undisturbed section at the samplng station to a

depth of approximately 15 cm. The core liners were removed carefully to minimize disturbance of the sediment-water interface. The core liners were capped to prevent loss of water and were placed upright in a wooden stand in order to sample the water. Two, 10 ml water samples were removed aseptically with a sterile 25 ml pipette and placed into sterile 16 x 125 mm screw top culture tubes. These samples were used to determine the number of viable A. hydrophila present in the water just above the sediment. The sediment samples were used to determine the chlorophyll$_a$ pigment concentration and organic content. The pigment was extracted in the dark at 4 C over a 48 hr period utilizing an 1 gm subsample and 50 ml of 90% acetone. The extraction samples were periodically shaken to resuspend the sediment and to facilitate the extraction process. The acetone supernatant was decanted into culture tubes, centrifuged for 10 min, and analyzed spectrophotometrically. Phytoplankton chlorophyll$_a$ concentration was determined from 350 ml of water passed through Gelman glass fiber filters (Type A/E, 0.45 micron pore size). The pigment was extracted in 90% acetone in the dark at 4 C for 48 hr prior to readings taken at the optical densities of 665, 645 and 630 nm (Richards and Thompson, 1952).

## Bacteriological Measurements

A reference type culture of A. hydrophila was obtained from the American Type Culture Collection (ATCC, E9071) to aid the identification of isolates from the natural habitat. The culture

was maintained on Tryptic Soy agar slants (Difco) and routinely grown in Tryptic Soy broth (Difco). Isolation, enumerations and characterization of A. hydrophila-like organisms from lake and river sediment were determined by the membrane filter technique of Rippey and Cabelli (1979) for Aeromonas spp. Small portions of the water samples were collected aseptically from the core liners and filtered through sterile membrane filters (pore size = 0.45 microns), placed on 5 ml Rippey-Cabelli (RC) differential agar plates and incubated for 20 hr at 37 C. The filters were examined for yellow pigmented colonies. Typical A. hydrophila colonies were circular, convex, yellow and 1 - 3 mm in diameter. These filters were transferred to a plate of mannitol agar medium and incubated for 2 to 3 hr at 37 C. Only the colonies that remained yellow (i.e. those that ferment mannitol) were counted. After the colonies were enumerated following the mannitol test, the filters were transferred to a filter pad saturated with phosphate-buffered saline (Levin and Cabelli, 1972) for 60 sec to partially neutralize the organic acid end products which obscure the color reaction of the oxidase test. The colonies were tested for cytochrome oxidase with 1% oxidase reagent (N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride) and positive oxidase colonies were assumed to be A. hydrophila-like organisms.

A 1 gm sediment sample was removed aseptically from the upper 1 cm of the sediment in the core liner with an alcohol-flamed stainless steel spatula and transferred into 99 ml of sterile

distilled water. These samples were shaken on a mechanical shaker for approximately 20 min to dislodge the bacteria. Ten-fold serial dilutions were made, filtered through 0.45 micron sterile membrane filters and plated on Rippey-Cabelli differential agar plates. The water and sediment samples were assayed within 8 hr after collection.

Periodically during the study colonies presumed to be A. hydrophila were stabbed into the medium developed by Kaper et al. (1979) as a check on the accuracy of the RC medium of Rippey and Cabelli (1979).

Enumeration of A. hydrophila from the Striped Mullet

Striped mullet were captured by electrofishing at night in the shallow shoreline region in Lake Harney. Skin mucous samples were taken using an alcohol-flamed plexiglass template with a window of 6 $cm^2$. Two skin samples for a total of 12 $cm^2$ were taken per fish. A sterile cotton swab was used to obtain the sample; it was placed into 4 ml of sterile phosphate-buffered saline (Levin and Cabelli, 1972). A 1 ml blood sample was taken aseptically by cardiac puncture with a 3.0 ml disposable plastic syringe equipped with a 3.8 cm, 22 guage stainless steel needle. The blood sample was transferred into 9 ml of sterile phosphate-buffered saline. The fish were transported on ice in an insulated cooler. At the laboratory, the sacrificed fish were processed within 8 hr for tissue samples of liver, kidney and spleen as well as for stomach

contents. Approximately 100 mg of the tissues and stomach contents were ground in 9.0 ml sterile distilled water in a sterile tissue grinder for 30 seconds. The skin mucous swab samples and the ground tissue samples were assayed for Aeromonas-like organisms and characterized by the sequential membrane filter procedure of Rippey and Cabelli (1979). In addition, chlorophyll$_a$ concentration of the stomach contents was determined. The chlorophyll$_a$ concentration of the stomach contents was determined spectrophotometrically from 100 mg samples using 90% acetone; extraction was conducted for 48 hr at 4 C in the dark. These samples were periodically shaken to resuspend the contents to insure maximum extraction. The dry weight of the stomach contents was determined in order that the chlorophyll$_a$ concentration could be expressed as mg Chl$_a$/gm dry wt stomach contents.

Transportation and Aquaria Surveys

Between April 27 and August 10, 1982 live specimens of striped mullet were obtained for laboratory observation from Lake Harney. They were captured by electrofishing on five occasions. Upon capture the fish were immediately placed under aeration in a live well on the electrofishing boat, transferred to a 760 l transport box containing aerated river water and transported to the laboratory. Striped mullet were observed in the laboratory in sterile 38 l aquaria containing pre-aerated tap water. Clear acrylic plastic lids were placed over the aquaria to minimize

aerosol effects of aeration. Water was drawn from the tanks through sterile tubing for the determination of dissolved oxygen, pH, turbidity and the enumeration of A. hydrophila-like bacteria. Changes in the external features of the fish were recorded every 3 hrs; these included the production of mucous, skin coloration changes and the response to an external stimulus. The aquaria observations were conducted over seven days. The water was exchanged in the aquaria every 24 hr.

Test Statistic

The degree of association of two variables is reported as Spearman's rank correlation coefficient (Ferguson, 1966); the rank correlation coefficient is determined from the equation: $r = 1 - (6 \Sigma d^2/n(n^2-1))$, where n is the number of paired observations $(X_i, Y_i)$ and $d = (X_i - Y_i)$.

## III. RESULTS AND DISCUSSION

Water depth at the sampling stations was at its lowest level in February, 1982; low water levels were found at each station (mean water depth was 0.33 m at both stations in February and March) until April 8, 1982 when the first major rain of the season increased water depth and flow. The water depth rose 1.04 m and the flow rate increased by thirteen times. Water depths tended to remain high throughout the remainder of the study (Tables I and II). Individual station values are presented in the Appendix. The mean water depth at Station 1 was 0.60 m (Table I) and 0.54 m at Station 2 (Table II). The monthly mean water temperature at Station 1 and Station 2 was 18.1 C and 21.5 C, respectively, in February, 1982, but increased to over 30 C at both stations by July, 1982.

Dissolved oxygen concentrations at the sampling stations were found to be highest at the beginning of the study. In February, dissolved oxygen was 7.8 mg/l at Station 1 and 8.3 mg/l at Station 2. Dissolved oxygen decreased sharply in March due to an increase in water temperature and tended to remain at low concentrations (3-4 mg/l) throughout the remainder of the study. The lowest monthly mean dissolved oxygen concentration occurred in April (2.1 mg/l) at Station 1 and occurred in August (1.6 mg/l) at Station 2. Relatively constant pH values were found at both stations, Tables I

and II. The pH ranged from 6.2 to 7.4 at Station 1 and from 6.6 to 7.5 at Station 2. The decrease in pH in April was attributed to incoming stormwater runoff at the beginning of the wet season. The decrease in pH that resulted was accompanied by a decrease in turbidity. Relatively higher turbidity values (24 - 62 NTU) were recorded at the sampling stations in February and March (Tables I and II). Turbidity tended to remain fairly low (2 - 4 NTU) after May, 1982. Total alkalinity ranged from 23.5 mg/l in April to 60.5 mg/l in February at Station 1. It was found to be only slightly lower at Station 2, Tables I and II. Minimum total alkalinty concentrations were recorded in April. This late winter decline in alkalinity coincided with an increase in rainfall and surface runoff. Maximum specific conductivity was found in February and March, 1982 with values approaching 1500 μmhos/cm @ 25 C. Minimum values for specific conductivity were recorded in August, Tables I and II. The specific conductivity generally was lower at Station 1 than at Station 2 throughout the study. A sharp decrease in specific conductivity was noted after the April rains.

The monthly mean phytoplankton chlorophyll$_a$ concentration was 47.8 mg/m$^3$ at both sampling stations in February, 1982. Soon after February, the chlorophyll$_a$ concentration decreased and tended to remain below 30 mg/m$^3$ throughout the remainder of the study, Table III. The higher chlorophyll$_a$ values in May and June can be attributed to algal blooms caused by the increase in water temperature and increase in nutrients in incoming runoff water from

Table I. Monthly mean values for physicochemical parameters taken at Underhill Slough (Station 1) in the St. Johns River from February through August, 1982.

| Month | Temperature (C) | Depth (m) | Turbidity (NTU) | Dissolved Oxygen (mg/1) | pH | Total Alkalinity (mg/1) | Specific Conductivity (μmhos/cm at 25 C) |
|---|---|---|---|---|---|---|---|
| February | 18.1 | 0.28 | 41 | 7.8 | 7.4 | 60.5 | 2962 |
| March | 24.8 | 0.37 | 24 | 3.9 | 6.6 | 31.5 | 1434 |
| April | 23.1 | 0.76 | 15 | 2.1 | 6.2 | 23.5 | 720 |
| May | 24.9 | 0.60 | 24 | 3.8 | 6.7 | 36.5 | 1396 |
| June | 29.4 | 0.52 | 2 | 3.2 | 6.6 | 33.2 | 1143 |
| July | 31.6 | 0.72 | 4 | 3.0 | 6.8 | 43.0 | 765 |
| August | 28.6 | 0.94 | 2 | 3.7 | 6.8 | 47.0 | 635 |
| Mean | 25.8 | 0.60 | 16 | 6.7 | 6.7 | 39.3 | 1294 |

Table II. Monthly mean values for physicochemical parameters at Cowhouse Slough (Station 2) in the St. Johns River from February through August, 1982.

| Month | Temperature (C) | Depth (m) | Turbidity (NTU) | Dissolved Oxygen (mg/l) | pH | Total Alkalinity (mg/l) | Specific Conductivity (µmhos/cm at 25 C) |
|---|---|---|---|---|---|---|---|
| February | 21.5 | 0.34 | 60 | 8.3 | 7.5 | 57.8 | 3148 |
| March | 25.6 | 0.33 | 62 | 5.8 | 6.9 | 56.8 | 3168 |
| April | 25.2 | 0.44 | 41 | 3.8 | 6.6 | 37.0 | 1712 |
| May | 26.9 | 0.56 | 6 | 7.0 | 7.2 | 44.2 | 2105 |
| June | 28.6 | 0.50 | 2 | 2.8 | 6.9 | 36.8 | 1380 |
| July | 31.5 | 0.78 | 2 | 3.2 | 6.8 | 49.0 | 780 |
| August | 28.8 | 0.85 | 3 | 1.6 | 6.7 | 46.5 | 565 |
| Mean | 26.9 | 0.54 | 25 | 4.6 | 6.9 | 46.9 | 1595 |

Table III. Monthly mean chlorophyll$_a$ concentrations in the water, sediment and stomach of striped mullet taken from the St. Johns River, February through August, 1982.

| Chlorophyll$_a$ | February | March | April | May | June | July | August |
|---|---|---|---|---|---|---|---|
| Water[a] | | | | | | | |
| Underhill Slough | 43.8 | 23.9 | 15.2 | 45.7 | 142.4 | 20.0 | 28.3 |
| Cowhouse Slough | 51.7 | 29.5 | 41.1 | 58.4 | 29.9 | 14.1 | 10.7 |
| Mean | 47.8 | 26.7 | 28.2 | 52.0 | 86.2 | 17.0 | 19.5 |
| Sediment[b] | | | | | | | |
| Underhill Slough | 15.5 | 18.3 | 16.1 | 14.3 | 20.8 | 9.5 | 10.2 |
| Cowhouse Slough | 28.6 | 11.6 | 13.1 | 23.4 | 24.7 | 16.2 | 23.0 |
| Mean | 22.0 | 15.0 | 14.6 | 18.8 | 22.8 | 12.8 | 16.6 |
| Stomach content[c] | 35.0 | | 59.1 | | 205.1 | | 63.5 |

[a] $mg/m^3$ of river water.

[b] mg/g dry weight of sediment.

[c] mg/g dry weight stomach content of striped mullet.

adjacent pasture land. The chlorophyll$_a$ concentration in the river was found to be strongly correlated with the chlorophyll$_a$ values in the sediment ($r = 0.262$, $P = 0.0005$). It also correlated with the chlorophyll$_a$ concentration found in the gut of striped mullet. In June, 1982 the maximum chlorophyll$_a$ concentration in striped mullet was found to be 205.1 mg/g dry weight which coincided with the highest maximum monthly mean chlorophyll$_a$ values found in the water and the sediment, Table III.

Monthly mean A. hydrophila-like densities in the river ranged from 0.26 to 5.64 x $10^3$ organisms/100 ml over the term of the study (Table IV). Maximum levels of the bacteria occurred in March, while minimum levels occurred in June. The monthly mean A. hydrophila-like density in the sediment reached a low level (0.72 x $10^3$ organisms/g dry weight of sediment) in June; this low density corresponded with the minimum density of bacteria found in the water (0.26 x $10^3$ organisms/100 ml). The bacteria in the sediment reached maximum density in August (8.78 x $10^3$ organisms/g dry weight) at the time of high bacteria levels in the water, Table IV. As one might expect, the density of A. hydrophila-like bacteria on the skin and in the gut of striped mullet was lowest in February. The number of A. hydrophila-like bacteria was highest in the gut of striped mullet in April (54.9 x $10^3$ organisms/g dry weight) and was highest on the skin of the fish in June (48 organisms/cm$^2$). The density of the bacteria in the sediment was weakly correlated with the abundance of the organism in the water ($r = 0.175$, $P = 0.10$).

Table IV. Monthly mean number of Aeromonas hydrophila-like organisms in the water, sediment and striped mullet at Underhill and Cowhouse Sloughs in the St. Johns River, February through August, 1982.

| Number of Organisms | February | March | April | May | June | July | August |
|---|---|---|---|---|---|---|---|
| Water[a] | | | | | | | |
| Underhill Slough | 1.32 | 4.80 | 4.03 | 1.08 | 0.46 | 0.98 | 2.45 |
| Cowhouse Slough | 0.69 | 6.47 | 0.31 | 1.25 | 0.06 | 0.58 | 0.50 |
| Mean | 1.01 | 5.64 | 2.17 | 1.17 | 0.26 | 0.78 | 1.48 |
| Sediment[b] | | | | | | | |
| Underhill Slough | 2.56 | 3.41 | 10.34 | 3.94 | 0.70 | 4.06 | 13.94 |
| Cowhouse Slough | 6.67 | 6.56 | 5.16 | 9.23 | 0.74 | 3.82 | 3.62 |
| Mean | 4.62 | 4.99 | 7.75 | 6.59 | 0.72 | 3.94 | 8.78 |
| Stomach Content[c] | 0.29 | | 54.90 | | 4.82 | | 2.42 |
| Skin[d] | 3.0 | | 25.0 | | 48.0 | | 4.0 |

[a] per 100 ml river water x $10^3$

[b] per g dry weight river sediment x$10^3$

[c] per g dry weight stomach content of striped mullet x $10^3$

[d] per $cm^2$ skin area on striped mullet

A correlation was not found between the abundance of the organism in the water and the density of the bacteria on the skin or in the gut of striped mullet.

The number of A. hydrophila-like bacteria was moderately correlated ($r = 0.8$, $P = 0.05$) with the chlorophyll$_a$ content in the gut of striped mullet. The bacteria was not found in the liver, spleen, kidney or blood of the fish at any time throughout the study. When the abundance of the bacteria in the water, sediment, gut and on the skin of the fish were compared to the physicochemical measurements on the river, the only linear correlations obtained were between the bacteria density in the water and the turbidity ($r = 0.351$, $P = 0.005$) and a negative relationship between the bacteria density in the water and water temperature ($r = -0.217$, $P = 0.10$). Correlations were not found between the bacteria density (water, sediment, gut or skin) with water depth, dissolved oxygen, pH, total alkalinity, specific conductivity or phytoplankton chlorophyll$_a$ concentration. Turbidity appeared to be primarily due to the abundance of phytoplankton. Rippey and Cabelli (1980) reported linear correlations between A. hydrophila levels in open water with chlorophyll$_a$ and Secchi disc transparency in lentic environments. While a similar result was anticipated in our study, the bacteria was not found to be correlated with chlorophyll$_a$. We had phytoplankton biomass values approximately three times higher than

reported by Rippey and Cabelli (1980). The lack of correlation between the density of the bacteria and the algal biomass may be that for eutrophic conditions where chlorophyll$_a$ is above 30 mg/m$^3$, a plateau for A. hydrophila levels may exist from the production of antimicrobial agents by algae (Rippey and Cabelli, 1980). Although bacteria levels fluctuated in the river throughout the study, the presence of lesions on striped mullet caused by A. hydrophila-like bacteria were seldom observed, Table IV.

Representative bacteria colonies identified as A. hydrophila-like organisms using the method of Rippey and Cabelli (1979) were periodically incubated in the stab medium developed by Kaper et al. (1979) to verify the presence of A. hydrophila and Enterobacteriaceae. The bacteria tested proved to be A. hydrophila in all these trials throughout the study.

The first transport trial of striped mullet from the St. Johns River consisted of 33 fish; in the laboratory these fish were placed in 100 l plastic containers containing 50 l of aerated water at a rate of 5 fish per container, Table V. The lips, fins, back and ventral surfaces of the mullet became reddened as the fish lost their ability to remain upright before becoming supine prior to death. All of the fish died within 48 hrs. Cultures from the skin of the fish and from the water did not confirm that death was caused by A. hydrophila-like bacteria. Since fish placed upon ice immediately after capture had similar symptoms as the transported

Table V. Survival of striped mullet in the laboratory after captured by electrofishing apparatus in the St. Johns River, April - August, 1982.

| Date | Number | Deaths | % Survival |
|---|---|---|---|
| April 26, 1982 | 33 | 33 | 0 |
| June 21 | 24 | 2 | 93 |
| June 29 | 41 | 3 | 93 |
| July 2 | 13 | 1 | 96 |
| July 26 | 27 | 0 | 100 |
| August 18 | 38 | 0 | 100 |

fish, this lead to the assumption that the immediate death of the transported fish was not caused by A. hydrophila-like bacteria, but perhaps was related to stress. Since hyperactivity and stress from capture may induce death (Black, 1958; Bouck and Ball, 1966), quinaldine was used to tranquilize transported fish; it was added at a concentration of 28 ppm to the transport water. In addition, the number of transported fish was kept at no more than one fish per 10 l of water to minimize the effect of crowding.

The second trial (June 21, 1982) consisted of 12 striped mullet placed into a 0.14 ha freshwater experimental pond on the campus of the University of Central Florida and 15 fish transported to the laboratory for observations in aquaria. The fish placed in the freshwater pond recovered from the quinaldine sedation in about 5 min. Daily observation of the pond did not reveal any death of these fish. Several of the 15 fish held in laboratory aquaria had a reddening of the lips and a slight reddening along the ventral surface. Thirteen of these fish became acclimated to the aquaria while the remaining two fish became extremely reddened and finally died. When the pond was drained three weeks later, the fish were recovered; they appeared healthy and lacked reddening and lesions. On three subsequent electrofishing trips to obtain striped mullet from the St. Johns River, the fish were captured and sedated in 28 ppm quinaldine. Of the 41 fish obtained on June 29, 1982, only three died. Sixty-five striped mullet captured on July 26 and August 18 had no mortality.

On July 8, 1982 twelve striped mullet were placed, individually, into laboratory aquaria, acclimated for nine days and then challenged with $10^1$, $10^2$, $10^3$, $10^4$ and $10^5$ A. hydrophila organisms/ml. The bacteria used were isolated from colonies obtained from the St. Johns River. In all cases, the fish appeared immune to the bacteria and did not become diseased. The dissolved oxygen concentration, pH and turbidity were monitored in the aquaria throughout the course of the experiment, Table VI. Little variation was noted for these parameters during the challenge.

Table VI. Mean dissolved oxygen, pH and turbidity of aquaria water during the challenge of striped mullet with A. hydrophila over a 36 hr on July 8-9, 1982.

| Parameter | 0 hr | 3 hr | 6 hr | 12 hr | 18 hr | 24 hr | 36 hr |
|---|---|---|---|---|---|---|---|
| Dissolved oxygen (mg/l) | 8.1 | 8.1 | 8.5 | 8.4 | 8.5 | 8.4 | 8.4 |
| Turbidity (NTU) | 0.6 | 3.2 | 3.2 | 3.1 | 3.0 | 3.3 | 3.7 |
| pH | 8.3 | 8.1 | 7.9 | 8.3 | 8.2 | 8.1 | 8.1 |

## IV. SUMMARY

*A. hydrophila*-like bacterial density in the water of the St. Johns River was correlated with turbidity and water temperature and with the density of the bacteria in the sediment. Linear correlations were not found between the density of the bacteria in the water with water depth, dissolved oxygen concentration, pH, total alkalinity, specific conductivity, or phytoplankton biomass as measured by chlorophyll$_a$ concentration. The pigment concentration of the phytoplankton was found to be correlated with chlorophyll$_a$ extracted from the river sediment. The bacteria found in the gut of striped mullet was significantly correlated with the chlorophyll$_a$ content in the stomach. The amount of bacteria on the skin of the fish was not found to be related to the number of bacteria in the water nor the appearance of red sores on the skin. Healthy striped mullet were captured and successfully transported under the influence of quinaldine during periods of high *A. hydrophila* density. The amount of stress (handling and crowding) appeared to be more significant for infection and death of the fish than the density of *A. hydrophila*. When the fish were challenged with extremely high densities of *A. hydrophila* in aquaria trials, they did not become diseased. The tranquilizing of the fish with 28 ppm quinaldine to reduce stress increased survival beyond 93%. We conclude that stress of the striped mullet enhances infection

from A. hydrophila during transport. This problem can be avoided by using a tranquilizer (quinaldine) and by reducing stress caused by crowding. Diseased striped mullet in the wild, from an infection of A. hydrophila, is probably related to environmental stress such as low dissolved oxygen concentration or a rapid change in water temperature.

# Appendix

Description of Contents:

Station values for physicochemical parameters, chlorophyll $a_a$ concentration and *Aeromonas hydrophila*-like organisms in the water, sediment and striped mullet.

Table VII. Physicochemical parameters taken at Underhill (Station 1) and Cowhouse (Station 2) Sloughs in the St. Johns River on February 19, 1982. Four replicate samples were taken/station/parameter.

| Station | Temperature (C) | Depth (m) | Turbidity (NTU) | Dissolved Oxygen (mg/1) | pH | Total Alkalinity (mg/1) | Specific Conductivity (µmhos/cm @ 25 C) |
|---|---|---|---|---|---|---|---|
| 1a | 18.0 | 0.30 | 40 | 7.6 | 7.3 | 61.0 | 2935 |
| 1b | 18.0 | 0.30 | 45 | 7.6 | 7.3 | 61.0 | 2935 |
| 1c | 18.2 | 0.25 | 40 | 7.8 | 7.4 | 60.0 | 2990 |
| 1d | 18.2 | 0.25 | 38 | 8.2 | 7.4 | 60.0 | 2990 |
| 2a | 20.4 | 0.34 | 57 | 8.0 | 7.3 | 58.0 | 3131 |
| 2b | 20.4 | 0.34 | 60 | 7.9 | 7.5 | 60.0 | 3131 |
| 2c | 22.7 | 0.34 | 63 | 8.6 | 7.5 | 57.0 | 3164 |
| 2d | 22.7 | 0.34 | 59 | 8.8 | 7.7 | 56.0 | 3164 |

Table VIII. Physicochemical parameters taken at Underhill (Station 1) and Cowhouse (Station 2) Sloughs in the St. Johns River on March 16, 1982. Four replicate samples were taken/station/parameter.

| Station | Temperature (C) | Depth (m) | Turbidity (NTU) | Dissolved Oxygen (mg/1) | pH | Total Alkalinity (mg/1) | Specific Conductivity (µmhos/cm @ 25 C) |
|---|---|---|---|---|---|---|---|
| 1a | 24.3 | 0.42 | 19 | 3.0 | 6.4 | 25.0 | 1004 |
| 1b | 24.3 | 0.42 | 15 | 3.0 | 6.4 | 26.0 | 1004 |
| 1c | 25.3 | 0.32 | 28 | 4.8 | 6.8 | 38.0 | 1863 |
| 1d | 25.3 | 0.32 | 32 | 4.9 | 6.7 | 37.0 | 1863 |
| 2a | 25.4 | 0.28 | 62 | 5.2 | 6.9 | 59.0 | 3258 |
| 2b | 25.4 | 0.28 | 65 | 5.1 | 6.9 | 61.0 | 3258 |
| 2c | 25.9 | 0.38 | 62 | 6.6 | 6.9 | 54.0 | 3078 |
| 2d | 25.9 | 0.38 | 58 | 6.5 | 6.9 | 53.0 | 3078 |

Table IX. Physicochemical parameters taken at Underhill (Station 1) and Cowhouse (Station 2) Sloughs in the St. Johns River on April 30, 1982. Four replicate samples were taken/station/parameter.

| Station | Temperature (C) | Depth (m) | Turbidity (NTU) | Dissolved Oxygen (mg/1) | pH | Total Alkalinity (mg/1) | Specific Conductivity (µmhos/cm @ 25 C) |
|---|---|---|---|---|---|---|---|
| 1a | 22.9 | 0.95 | 13 | 2.0 | 6.1 | 22.0 | 623 |
| 1b | 22.9 | 0.95 | 17 | 2.0 | 6.1 | 22.0 | 623 |
| 1c | 23.3 | 0.58 | 16 | 2.1 | 6.3 | 25.0 | 818 |
| 1d | 23.3 | 0.58 | 15 | 2.3 | 6.3 | 25.0 | 818 |
| 2a | 25.2 | 0.47 | 46 | 4.2 | 6.6 | 39.0 | 1664 |
| 2b | 25.2 | 0.47 | 37 | 4.2 | 6.6 | 38.0 | 1664 |
| 2c | 25.1 | 0.40 | 40 | 3.4 | 6.6 | 36.0 | 1761 |
| 2d | 25.1 | 0.40 | 42 | 3.5 | 6.6 | 35.0 | 1761 |

Table X. Physicochemical parameters taken at Underhill (Station 1) and Cowhouse (Station 2) Sloughs in the St. Johns River on May 17, 1982. Four replicate samples were taken/station/parameter.

| Station | Temperature (C) | Depth (m) | Turbidity (NTU) | Dissolved Oxygen (mg/1) | pH | Total Alkalinity (mg/1) | Specific Conductivity (µmhos/cm @ 25 C) |
|---|---|---|---|---|---|---|---|
| 1a | 25.5 | 0.54 | 8 | 4.0 | 6.8 | 37.0 | 1083 |
| 1b | 25.5 | 0.54 | 20 | 3.6 | 6.7 | 35.0 | 1083 |
| 1c | 24.2 | 0.66 | 8 | 3.9 | 6.8 | 37.0 | 1710 |
| 1d | 24.2 | 0.66 | 60 | 3.7 | 6.7 | 37.0 | 1710 |
| 2a | 27.2 | 0.52 | 6 | 7.0 | 7.2 | 45.0 | 2070 |
| 2b | 27.2 | 0.52 | 6 | 7.2 | 7.2 | 44.0 | 2070 |
| 2c | 26.6 | 0.52 | 5 | 6.9 | 7.2 | 44.0 | 2140 |
| 2d | 26.6 | 0.52 | 7 | 6.9 | 7.2 | 44.0 | 2140 |

Table XI. Physicochemical parameters taken at Underhill (Station 1) and Cowhouse (Station 2) Sloughs in the St. Johns River on June 16, 1982. Four replicate samples were taken/station/parameter.

| Station | Temperature (C) | Depth (m) | Turbidity (NTU) | Dissolved Oxygen (mg/1) | pH | Total Alkalinity (mg/1) | Specific Conductivity (µmhos/cm @ 25 C) |
|---|---|---|---|---|---|---|---|
| 1a | 29.8 | 0.41 | 2 | 3.1 | 5.9 | 19.0 | 1140 |
| 1b | 29.8 | 0.41 | 1 | 2.7 | 6.8 | 38.0 | 1140 |
| 1c | 29.0 | 0.62 | 2 | 3.7 | 6.8 | 37.0 | 1146 |
| 1d | 29.0 | 0.62 | 1 | 3.2 | 6.8 | 39.0 | 1146 |
| 2a | 28.9 | 0.40 | 2 | 2.5 | 6.8 | 39.0 | 1330 |
| 2b | 28.9 | 0.40 | 3 | 2.5 | 6.9 | 28.0 | 1330 |
| 2c | 28.4 | 0.59 | 1 | 3.0 | 7.0 | 39.0 | 1430 |
| 2d | 28.4 | 0.59 | 3 | 3.1 | 6.8 | 41.0 | 1430 |

Table XII. Physicochemical parameters taken at Underhill (Station 1) and Cowhouse (Station 2) Sloughs in the St. Johns River on July 12, 1982. Four replicate samples were taken/station/parameter.

| Station | Temperature (C) | Depth (m) | Turbidity (NTU) | Dissolved Oxygen (mg/1) | pH | Total Alkalinity (mg/1) | Specific Conductivity (µmhos/cm @ 25 C) |
|---|---|---|---|---|---|---|---|
| 1a | 31.3 | 0.72 | 5 | 2.6 | 6.4 | 36.0 | 700 |
| 1b | 31.3 | 0.72 | 4 | 2.5 | 6.8 | 41.0 | 700 |
| 1c | 31.8 | 0.72 | 2 | 3.5 | 7.0 | 47.0 | 830 |
| 1d | 31.8 | 0.72 | 3 | 3.4 | 6.9 | 48.0 | 830 |
| 2a | 31.6 | 1.00 | 2 | 3.3 | 6.8 | 52.0 | 760 |
| 2b | 31.6 | 1.00 | 1 | 3.2 | 6.8 | 48.0 | 760 |
| 2c | 31.4 | 0.55 | 3 | 3.0 | 6.8 | 49.0 | 800 |
| 2d | 31.4 | 0.55 | 1 | 3.1 | 6.9 | 47.0 | 800 |

Table XIII. Physicochemical parameters taken at Underhill (Station 1) and Cowhouse (Station 2) Sloughs in the St. Johns River on August 4, 1982. Four replicate samples were taken/station/parameter.

| Station | Temperature (C) | Depth (m) | Turbidity (NTU) | Dissolved Oxygen (mg/1) | pH | Total Alkalinity (mg/1) | Specific Conductivity (µmhos/cm @ 25 C) |
|---|---|---|---|---|---|---|---|
| 1a | 28.5 | 0.70 | 0 | 2.1 | 6.7 | 46.0 | 620 |
| 1b | 28.5 | 0.70 | 4 | 2.1 | 6.7 | 47.0 | 620 |
| 1c | 28.7 | 1.19 | 3 | 5.2 | 6.8 | 47.0 | 650 |
| 1d | 28.7 | 1.19 | 2 | 5.3 | 6.8 | 48.0 | 650 |
| 2a | 28.9 | 0.75 | 5 | 1.5 | 6.7 | 46.0 | 500 |
| 2b | 28.9 | 0.75 | 4 | 1.5 | 6.7 | 46.0 | 500 |
| 2c | 28.8 | 0.95 | 2 | 1.8 | 6.7 | 47.0 | 630 |
| 2d | 28.8 | 0.95 | 2 | 1.7 | 6.7 | 47.0 | 630 |

Table XIV. Monthly numbers of Aeromonas hydrophila-like organisms/100 ml of lake and river water samples taken in Underhill (Station 1) and Cowhouse (Station 2) Sloughs in the St. Johns River from February through August, 1982.

| | Aeromonas hydrophila (number/100 ml x $10^3$) | | | | | | |
|---|---|---|---|---|---|---|---|
| Station | February | March | April | May | June | July | August |
| 1a | 2.63 | 5.03 | 2.64 | 1.44 | 0.00 | 0.99 | 7.00 |
| 1b | 0.33 | 3.22 | 3.72 | 0.00 | 0.44 | 1.16 | 2.00 |
| 1c | 0.11 | 6.04 | 7.78 | 1.67 | 0.61 | 0.84 | 0.80 |
| 1d | 2.22 | 4.92 | 2.17 | 1.22 | 0.78 | 0.90 | 0.00 |
| 2a | 0.61 | 7.31 | 1.11 | 2.11 | 0.00 | 0.83 | 0.10 |
| 2b | 1.11 | 6.78 | 0.22 | 0.00 | 0.00 | 0.58 | 0.80 |
| 2c | 0.22 | 6.56 | 0.11 | 1.56 | 0.00 | 0.54 | 0.30 |
| 2d | 0.39 | 5.22 | 0.00 | 1.33 | 0.22 | 0.34 | 0.80 |

Table XV. Monthly numbers of Aeromonas hydrophila-like organisms/gram dry weight of sediment samples taken in Underhill (Station 1) and Cowhouse (Station 2) Sloughs in the St. Johns River from February through August, 1982.

| | Aeromonas hydrophila (number/g dry weight sediment x $10^3$) | | | | | | |
|---|---|---|---|---|---|---|---|
| Station | February | March | April | May | June | July | August |
| 1a | 3.41 | 0.00 | 0.45 | 5.98 | 0.00 | 3.27 | 4.69 |
| 1b | 3.08 | 1.69 | 14.94 | 5.89 | 0.21 | 0.00 | 8.94 |
| 1c | 3.73 | 11.97 | 24.42 | 3.32 | 2.12 | 1.45 | 21.17 |
| 1d | 0.01 | 0.00 | 1.57 | 0.58 | 0.47 | 11.41 | 20.98 |
| 2a | 4.85 | 14.72 | 10.69 | 15.89 | 0.00 | 4.00 | 0.00 |
| 2b | 15.37 | 10.22 | 2.14 | 11.51 | 0.00 | 6.32 | 7.75 |
| 2c | 2.73 | 0.16 | 0.44 | 0.09 | 2.62 | 3.37 | 4.94 |
| 2d | 3.71 | 1.15 | 7.36 | 9.44 | 0.32 | 1.60 | 1.80 |

Table XVI. Number of Aeromonas hydrophila-like organisms/g dry weight stomach contents of striped mullet captured near Underhill Slough (Station 1) from February through August, 1982.

| Aeromonas hydrophila (number/g dry weight stomach content x $10^3$) | | | | |
|---|---|---|---|---|
| Fish | February | April | June | August |
| 1 | 0.26 | 34.40 | 4.38 | 0.00 |
| 2 | 0.28 | 6.55 | 5.22 | 0.00 |
| 3 | 0.34 | 102.00 | 9.99 | 7.86 |
| 4 | | | | 0.00 |
| 5 | | 59.10 | 0.91 | 0.00 |
| 6 | | 101.00 | 0.00 | 5.95 |
| 7 | | | 2.89 | 5.60 |
| 8 | | 59.10 | | 2.80 |
| 9 | | | 10.70 | 2.02 |
| 10 | | 22.00 | | 0.00 |

Table XVII. Number of Aeromonas hydrophila-like organisms/$cm^2$ of skin area of striped mullet captured near Underhill Slough (Station 1) from February through August, 1982.

| | Aeromonas hydrophila Organisms/$cm^2$ Skin Area | | | |
|---|---|---|---|---|
| Fish | February | April | June | August |
| 1 | 3 | 0 | 40 | 7 |
| 2 | 7 | 0 | 7 | 0 |
| 3 | 0 | 50 | 160 | 0 |
| 4 | | 23 | 40 | 10 |
| 5 | | 7 | 33 | 10 |
| 6 | | 23 | 13 | 0 |
| 7 | | 20 | 38 | 3 |
| 8 | | 93 | 13 | 10 |
| 9 | | 13 | 13 | 3 |
| 10 | | 20 | 127 | 0 |

Table XVIII. Chlorophyll$_a$ pigment concentration of lake and river water in Underhill (Station 1) and Cowhouse (Station 2) Sloughs in the St. Johns River from February through August, 1982.

| Station | Chlorophyll$_a$ Pigment Concentration (mg/m$^3$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | February | March | April | May | June | July | August |
| 1a | 47.8 | 14.3 | 15.4 | 52.0 | 137.4 | 15.8 | 34.6 |
| 1b | 46.0 | 16.0 | 15.1 | 44.1 | 131.1 | 27.8 | 35.9 |
| 1c | 40.4 | 34.4 | 14.3 | 47.4 | 145.1 | 18.9 | 21.7 |
| 1d | 41.2 | 31.0 | 15.9 | 39.4 | 156.1 | 17.4 | 20.9 |
| 2a | 51.2 | 27.1 | 40.9 | 61.0 | 22.5 | 12.6 | 16.5 |
| 2b | 46.7 | 29.4 | 41.7 | 59.3 | 21.4 | 11.7 | 14.2 |
| 2c | 52.7 | 33.4 | 44.9 | 56.5 | 37.5 | 15.6 | 6.2 |
| 2d | 56.1 | 28.0 | 37.0 | 56.8 | 38.3 | 16.4 | 6.0 |

Table XIX. Chlorophyll$_a$ pigment concentration of sediment samples taken in Underhill (Station 1) and Cowhouse (Station 2) Sloughs in the St. Johns River from February through August, 1982.

| Station | Chlorophyll$_a$ Pigment Concentration/g Dry Weight Sediment | | | | | | |
|---|---|---|---|---|---|---|---|
| | February | March | April | May | June | July | August |
| 1a | 14.7 | 14.5 | 1.0 | 18.9 | 16.1 | 9.0 | 7.6 |
| 1b | 19.6 | 20.0 | 32.2 | 9.5 | 9.8 | 6.7 | 11.6 |
| 1c | 14.0 | 14.2 | 15.8 | 15.9 | 25.5 | 6.0 | 13.6 |
| 1d | 13.6 | 24.8 | 15.3 | 12.8 | 31.8 | 16.2 | 7.9 |
| 2a | 36.7 | 8.6 | 7.3 | 30.4 | 2.5 | 7.2 | 29.5 |
| 2b | 28.8 | 15.0 | 8.2 | 25.5 | 9.3 | 15.0 | 23.7 |
| 2c | 21.8 | 10.2 | 17.5 | 7.0 | 39.4 | 15.0 | 21.1 |
| 2d | 27.0 | 12.5 | 19.6 | 30.9 | 47.5 | 27.6 | 17.6 |

Table XX. Chlorophyll$_a$ pigment concentration/g dry weight stomach content of striped mullet captured near Underhill Slough (Station 1) from February through August, 1982.

| Fish | Chlorophyll$_a$ Pigment Concentration (mg/g dry wt of stomach contents) | | | |
|---|---|---|---|---|
| | February | April | June | August |
| 1 | | 67.4 | 85.5 | 23.2 |
| 2 | 62.1 | 143.8 | 424.1 | 92.6 |
| 3 | 7.9 | 31.6 | 428.0 | 46.5 |
| 4 | | | | 37.2 |
| 5 | | 73.1 | 164.3 | 69.8 |
| 6 | | 22.7 | 51.5 | 144.2 |
| 7 | | | 198.4 | 74.2 |
| 8 | | 43.3 | | 64.0 |
| 9 | | | 84.0 | 27.4 |
| 10 | | 32.1 | | 55.9 |

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